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Mutations in the *MEFV* Gene in a Large Series of Patients With a Clinical Diagnosis of Familial Mediterranean Fever

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Familial Mediterranean fever (FMF) is an autosomal recessively inherited disease affecting patients of the Mediterranean basin. FMF is characterized by recurrent episodes of fever accompanied with topical signs of inflammation. Some patients can develop a renal amyloidosis associated (AA) amyloidosis. The administration of colchicine is an effective preventive treatment of both the attacks and amyloidosis. The FMF gene (*MEFV*) was cloned and missense mutations were found to be responsible for the disease. We investigated a large series of 303 unselected and unrelated patients of various ethnic backgrounds with a clinical suspicion of FMF to confirm or invalidate the diagnosis of FMF and to determine the spectrum of *MEFV* mutations. Molecular analysis focused on all the most frequent mutations identified so far, and an exhaustive analysis of exon 10, containing the mutational hotspot, was performed through DNA sequencing. Sixty-two percent of Sephardic, North African Arabs, Armenian and Turkish patients were either homozygous or compound heterozygous for *MEFV* mutations. In other populations surrounding the Mediterranean Sea such as Greek, Italian, Portuguese, Kurdish and Lebanese populations, mutations were also found. In general, patients without Mediterranean origin had no mutations in the *MEFV* gene. Two new missense mutations were identified in exon 10 of the *MEFV* gene: the S675N in an Italian patient and the M680L in a French patient without any known at-risk ethnic ancestry. Am. J. Med. Genet. 92:241–246, 2000.

KEY WORDS: familial Mediterranean fever; FMF; missense mutations; *MEFV* gene.

INTRODUCTION

Familial Mediterranean fever (FMF, MIM 249100) is an autosomal recessive disorder of the inflammatory pathway [Drenth et al., 1994]. FMF is the most frequent disease among the group of hereditary fevers including the hyperimmunoglobulinemia D with periodic fever syndrome (MIM 260920) [Ben Chetrit and Levy, 1998; Grateau et al., 1999; Drenth et al., 1999; Houten et al., 1999] and the group of autosomal dominant recurrent fevers (MIM 142680 and 134610) [McDermott et al., 1997, 1999]. FMF is characterized by recurrent episodes of fever accompanied by topical signs of inflammation mainly in the peritoneal, pleural and articular cavities. The main complication of the disease is the occurrence of renal amyloidosis of the AA (amyloidosis associated) type [Sohar et al., 1967]. FMF affects populations of the Mediterranean basin such as North African Arabs, Armenian, Sephardic Jewish and Turkish populations. Carrier frequency has been estimated to reach 1:7 to 1:5 in Armenians and Sephardic Jews, respectively [Rogers et al., 1989; Daniels et al., 1995]. The high frequency of mutation in *MEFV* suggests that heterozygotes may have some selective advantages, for example increased resistance to an unidentified agent. Given the absence of pathognomonic clinical symptoms, the diagnosis of FMF remains one of exclusion [Livneh et al., 1997]. It can be very difficult to establish in the following situation: atypical clinical signs, late onset beginning, absence of family history or ethnic background. The clinical diagnosis of FMF, however, leads to the beginning of a daily and life-long

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administration of colchicine that is an efficient preventive treatment of both the attacks and amyloidosis [Zemer et al., 1986]. The gene responsible for FMF (designated *MEFV*), located on the short arm of chromosome 16 (16p13.3) [Pras et al., 1992], has been identified by positional cloning [French FMF Consortium 1997; International FMF Consortium, 1997]. The *MEFV* transcript is expressed in granulocytes that play an essential role in the inflammatory response. The *MEFV* gene encodes a protein of 781 residues named marenostrin [French FMF Consortium, 1997] or pyrin [International FMF Consortium, 1997]. The presence of nuclear localization signals (NLS) [Robbins et al., 1991] a leucine zipper domain and a basic region suggests that marenostrin/pyrin functions as a nuclear effector molecule through homomultimerization [French FMF Consortium, 1997; International Consortium, 1997]. Four missense mutations were found in these studies. Some additional mutations have been discovered since then [Bernot et al., 1998; Samuels et al., 1998; Booth et al., 1998; Aksentijevich et al., 1999; Cazeneuve et al., 1999]. We prospectively investigated a large series of unselected and unrelated patients of various ethnic backgrounds with a clinical suspicion of FMF to determine the spectrum of the mutations and to confirm or invalidate the diagnosis of FMF.

MATERIAL AND METHODS

Patients

After the discovery of the *MEFV* gene, the routine molecular diagnosis of FMF in our laboratory began in November 1997. The *MEFV* genotypes of 303 patients in whom FMF was suspected on a clinical basis have been determined [Livneh et al., 1997]. Main clinical data were registered on a standardized form: age, gender, origin of both parents, familial consanguinity, history of FMF, age at onset of the inflammatory attacks, duration of attacks, organ involvement, joint pain, chronic joint disease, frequency of the attacks, splenomegaly, amyloidosis and efficiency of colchicine. When a compound heterozygosity was found in a patient, we determined the genotypes of the parents to find out if the mutations were situated on the same or on different chromosomes.

Methods

DNA extraction. Genomic DNA was isolated from the patients' peripheral-blood leukocytes using standard procedures [Sambrook et al., 1989].

Mutation analysis. The region of exon 10 covering codons 663 to 771 was amplified using the following primers: 5′-GAGGTGGAGGTTGGAGACAA-3′ and 5′-TGACCACCCACTGGACAGAT-3′. The 50 μl reaction was carried out using Boehringer manufacturer's conditions with 2.5 mM $MgCl_2$, 250 μM of each dNTP, 0.2 μM of each primer, 1.5 U of Taq polymerase as follows: 94°C for 4 min followed by 35 cycles of denaturation 94°C (45 sec), annealing 53°C (30 sec) and extension at 72°C (30 sec) with a final 4 min extension at 72°C. The PCR products were then purified using Microcon 100 (Amicon) filtration system, sequencing reactions were carried out using the ABI PRISM dye terminator cycle sequencing kit, and the products were analyzed on an ABI 377 DNA sequencer. DNA sequencing was performed in both directions, initiated from the forward and the reverse primers used in the initial PCR.

The following PCR primers: 5′-AACTTTAATATCCAAGGGGATTC-3′ and 5′TTCTCTGCAGCCGATATAAAGTA-3′ were used for amplification of exon 2 in the same conditions as above except the presence of 10% DMSO. The G→C transversion at codon 148 was analyzed as previously described [Bernot et al., 1998]. The P369S mutation (CCC→TCC) was analyzed using the modified forward primer 5′-GAAGAGCCCGGGAAGCCTGAGC-3′ creating a SacI restriction site when the mutation is present and the reverse primer 5′-TTGGGAAAATGAAGTAAGGCC-3′ [Cazeneuve et al., 1999]. The digestions were then analyzed on a 4% low melting point agarose gel. Mutations in exon 5 were screened by DGGE technique as described previously [Cazeneuve et al., 1999].

RESULTS

The main clinical characteristics of the 303 patients were the following: fever was observed in 254 patients (83.3%), abdominal signs in 225 (74.0%), thoracic signs in 73 (24%), joint signs in 153 (50.3%), erysipela-like erythema in 25 (8.2%), splenomegaly in 26 (8.5%), amyloidosis in 12 (4%). The age at onset of the disease was before 30 in 84% of the patients. Among the patients studied, 31% were Sephardic Jews, 10% Armenians, 16% Arabs, 8% Turks, 9% French without typical ethnic background and 26% belonged to other ethnic groups or only one parent belonged to an at-risk ethnic group.

The results of the different genotypes found in the patients are shown in Table I. The patients were divided into two groups: in the first one, including patients from Sephardic Arab, Armenian, and Turkish populations, 62% (122/197) were homozygous or compound heterozygous for *MEFV* mutations. In the second group, including patients of French or other non at-risk population origins and patients who had only one parent belonging to an at-risk ethnic group, only 10.3% (11/106) of the patients carried two *MEFV* mutations. Eighteen different genotypes were characterized among 133 patients carrying two mutations. The greatest diversity of genotypes was observed among Armenians and Turks with 10 different genotypes for 21 patients and 7 different genotypes for 24 patients, respectively. Most of them were compound heterozygotes. The Sephardic population was more homogenous with a main genotype M694V/M694V in 47/93 patients (50.5%). One Sephardic patient was homozygous for the E148Q mutation without mutations in exon 10 and without the P369S mutation. In the Arab population, two genotypes were found to be strongly overrepresented, the M694I/M694I (24.5%) and the M694V/M694V (14.2%) respectively. Because there were only 2 heterozygotes among 49 patients, the number of mutations in this population is probably limited. The M694V/R761H genotype has been observed twice, in a Syrian and in a Kurdish patient, respectively. In all cases studied the genotype analysis of parents of pa-

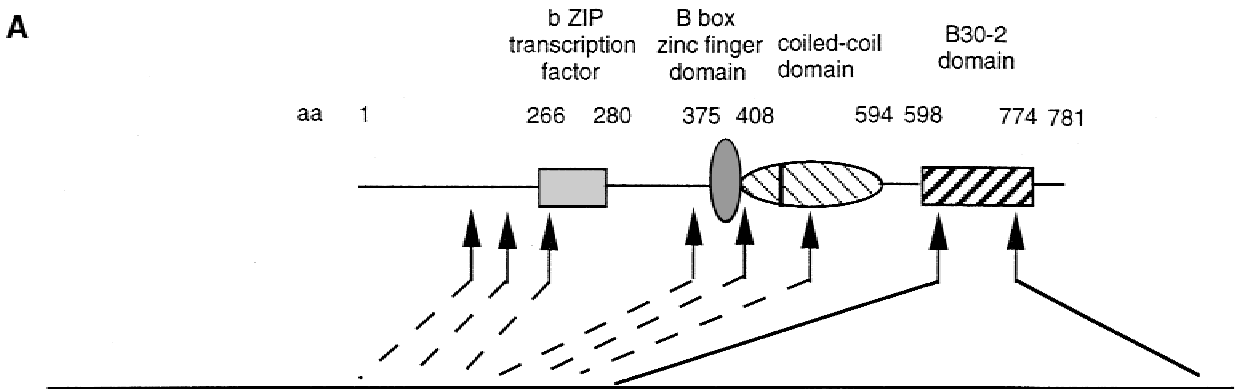
TABLE I. Genotype Distribution in the Different Ethnic Groups

	At-risk populations				Non at-risk populations		
Genotypes	Sephardic Jews	Arabs	Armenians	Turks	French	Other populations*	Total
Homozygotes							
E148Q/E148Q	1						
M680I/M680I			2				
M694I/M694I		12				1[a]	
M694V/M694V	47	7	4	8			
V726A/V726A			1	1		1[b]	
Total	48	19	7	9		2	85
Compound heterozygotes							
E148Q/M680I				1			
E148Q/M694V	3		2	1		1[c]	
						1[d]	
P369S/M694V	1						
F479L/V726A			1				
M680I/M694I		1	1				
M680I/M694V		1	6	1		1[c]	
						1[d]	
M680I/V726A			5	2			
M694I/M694V		1					
M694I/V726A						1[e]	
M694V/R761H		1				1[f]	
M694V/V726A	3		5	2		2[g]	
KG95R/V726A						1[g]	
Total	7	4	20	7		9	47
Heterozygotes							
E148Q-P369S						1[h]	
E148Q	4	2		3	1	1[c]	
S675N						1[i]	
M680L					1		
M680I			1				
M692del						1[c]	
M694I						1[a]	
						1[i]	
M694V	16			1		1[j]	
						2[d]	
						3[k]	
						1[i]	
						2[l]	
K695R	1					1[d]	
Total	21	2	2	4	2	16	47
Unidentified	17	24	2	4	26	51	124
Total	93	49	31	24	28	78	303

*The ethnic origin of both parents has been precised.
[a]Portuguese/Portuguese.
[b]Ashkenazi/Ashkenazi.
[c]Lebanese/Lebanese.
[d]Jewish/?.
[e]Jewish/Arab.
[f]Kurdish/Kurdish.
[g]Sephardic/Ashkenazi.
[h]Ashkenazi/?.
[i]Italian/Italian.
[j]Spanish/French.
[k]Greek/Greek.
[l]?/?.

tients with compound heterozygosity showed that the mutations were situated on different alleles. Among these parents who were over 35 years old, 3 were found to be compound heterozygotes (2 M694V/V726A and 1 M680I/V726A), and remained asymptomatic at the date of the study (data not shown). In the French population, no patient were found homozygotes or compound heterozygotes for *MEFV* mutations.

The locations of the different mutations in the marenostrin/pyrin, published until now, and the frequency of the different mutated alleles in each ethnic group found in our series are shown in Figure 1. Thirteen different mutations were found in the B30.2 domain, nine of which (including 2 novel ones) represented 94.6% of the mutated alleles found in our series. In the Sephardic Jews our data are similar to previous data, the M694V mutation being strongly predominant (91%). Rare mutations, such as E148Q, P369S, K695R and V726A, could possibly be inherited from Ashkenazim Jews through migrations in Europe and mixed



B

	E148Q	E167D	T267I	P369S	R408Q	F479L	S675N	M680I (C)	M680I (T)	M680L	T681I	I692del	M694V	M694I	M694del	K695R	V726A	A744S	R761H
Sephardic Jews(n=131)	7%			<1%									91%			<1%	2%		
Armenians (n=57)	5%			<2%		<2%		30%					28%	10.5%			23%		
Arabs (n=48)	4%							4%					35%	54%					<2%
Turks (n=36)	14%							11%					58.5%				16.5%		

C

Ashkenazi Jews																	2		
Italians							1												
Kurdish													1						1
Libaneses	2							1				1	1	1					
Portugueses														3					
French										1									

Fig. 1. Mutation spectrum in the *MEFV* gene. (**A**) The different characteristic domains of the marenostrin/pyrin gene. Mutations in bold are those found in our study. (**B**) Mutation frequency in at-risk ethnic groups in our series (n = number of mutated alleles). (**C**) Rare mutation alleles found in populations living in the Mediterranean basin, in our series.

unions with the Sephardic population [Aksentijevich et al., 1999]. In Turks, our results are in agreement with those of Chen et al .,[1998] who found three main mutations M694V (58%), V726A (16.5%) and M680I (10.5%) accounting for 29 of the 34 disease alleles in 16 families. The greatest variety of different mutations was found in patients of Armenian origin; the most common being M680I (30%), M694V (24%) V726A (23%), M694I (10.5%), E148Q (5%). In the Arab population, mainly originating from North Africa, the M694I and the M694V mutations predominated (54% and 35%, respectively). Other rare mutations such as M680I, E148Q and R761H were also observed in the North African Arab population. Two unrelated patients from Portugal had the M694I mutation. Two new heterozygous mutations were observed in exon 10, the first one S675N (AGC→AAC) in an Italian patient and the second one M680L (ATG→CTG) in a French patient (Fig. 2). The rare mutations A744S, T681I, I692del and R408Q were not found in this study.

DISCUSSION

Our strategy for the molecular diagnosis of FMF is based on exon 10 sequencing after PCR amplification of genomic DNA as most of the *MEFV* mutations are clustered in this region. Although other strategies based on PCR amplification such as Amplification Refractory Mutation Strategy (ARMS) or restriction analysis are accurate [Cazeneuve et al., 1999; Eisenberg et al., 1998], sequencing allows the detection of all known and potential new mutations in the region of exon 10 in a single-step reaction. Two other frequent mutations, E148Q and P369S, in exons 2 and 3 respectively, were analyzed by restriction enzyme analysis. Very rare mutations such as E167D and T267I in exon 2 [Bernot et al., 1998] and R408Q in exon 3 [Cazeneuve et al., 1999], described only once, were not investigated in this study.

As we did not identify two mutations in all patients belonging to at-risk ethnic groups, this suggests either the presence of mutations in other exons of *MEFV* or

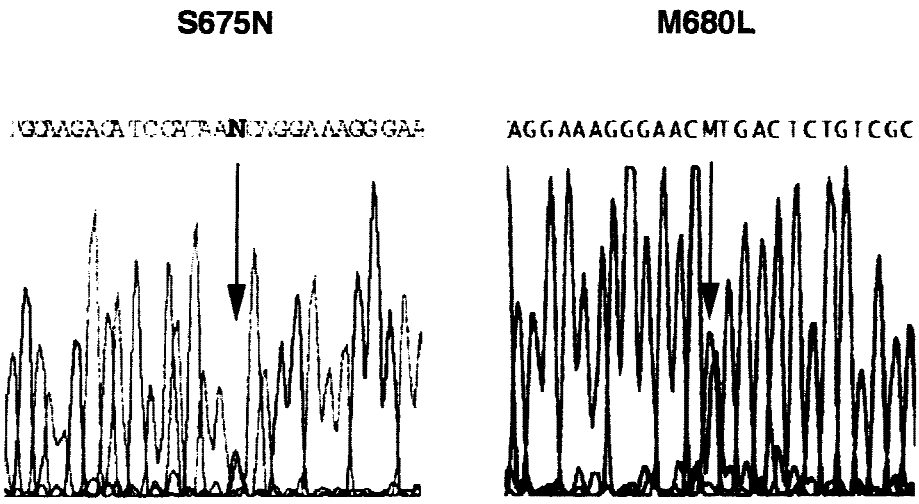


Fig. 2. Sequence analysis of exon 10 in two different FMF patients. The arrows point to heterozygous alteration. The G→A mutation changes the sequence of codon 675 from AGC to AAC and results in a predicted serine-to-asparagine substitution. The A→C mutation changes the sequence of codon 680 from ATG to CTG and results in a predicted methionine-to-leucine substitution.

that another possible FMF locus, not yet located, can exist as suggested in the Turkish population [Akarsu et al., 1997] that could account for the genetic heterogeneity.

We also identified mutations in patients belonging to other ethnic groups such as Greeks, Italians, Portuguese. Even in our population of French origin with no known Mediterranean ancestry, we found an ATG→CTG mutation at position 680 leading to the substitution of methionine with leucine. This mutation was not detected in 200 control alleles, making it unlikely that it represented a common polymorphism. We recently found one Moroccan patient (not included in this series) who is homozygous for the M680L mutation. The codon 680 seemed to be a hot-spot for mutation because two mutations were previously described modifying the methionine to isoleucine (ATG→ATA or ATC). Only one new sequence variation, M694del, has until now been reported in an English patient with typical FMF and without Mediterranean ancestry [Booth et al., 1998].

The large number of patients (46/303, 15%) with only one mutation, despite the search for new mutations such as P369S, makes the interpretation of this status difficult in populations where the frequency of the carrier of one copy of the gene is considered to be between 1/10 and 1/5 [Rogers et al., 1989; Daniels et al., 1995]. This finding suggests that other mutations remain to be discovered in other regions of the *MEFV* gene, but it cannot be excluded that heterozygotes may present mild or sometimes atypical signs of FMF. In populations where the carrier frequency is much lower, although unknown, such as Italians, Greeks or Portuguese, the discovery of only one mutation in a patient has probably a much higher diagnostic value for FMF.

Three parents of patients, still asymptomatic at 35 years or older, were found to be compound heterozygotes, sharing the mild V726A mutation. These data raise the question of the treatment with colchicine in this situation.

We conclude that the diagnosis of FMF should not be done only on the basis of clinical criteria and should include molecular studies. This is a very important point for patients at the beginning of the disease or without family history, to know if a daily long-life colchicine therapy should be started or not.

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